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COMMENTARY

CATECHOLAMINE SYNTHESIS: PHYSIOLOGICAL COUPLING TO PRECURSOR SUPPLY

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A decade ago, *Biochemical Pharmacology* published a Commentary article on the "Control of Brain Neurotransmitter Synthesis by Precursor Availability and Nutritional State" [1]. At that time, considerable evidence was available that low doses of exogenous tryptophan, which elevated brain tryptophan but kept its levels within their normal range, could increase brain serotonin concentrations and those of serotonin's metabolite 5-hydroxyindoleacetic acid [2]. Moreover, food consumption had already been shown to influence brain tryptophan, and thereby brain serotonin levels, with carbohydrate-rich meals increasing [3] and protein meals decreasing [4] production of indoles, and it had been proposed that this coupling of food composition to serotonin synthesis allows serotonin-dependent behaviors to be affected by eating [5, 6].

It was also then known that consumption of supplemental tyrosine [7] or choline [8, 9] could affect the syntheses of their neurotransmitter products, the catecholamines and acetylcholine. However, these responses were poorly characterized, and were observed to be considerably less consistent than that of serotonin to supplemental tryptophan: tyrosine administration only sometimes increased brain levels of catecholamine metabolites, and even then it failed to affect those of dopamine or norepinephrine; choline administration only sometimes affected brain acetylcholine levels [10], and there were no acetylcholine metabolites to measure as evidence that more acetylcholine molecules were turning over. Moreover, no direct evidence was available that administration of tyrosine or choline could enhance either the release of their neurotransmitter products or the postsynaptic responses to the transmitters.

This article comments on information accumulated during the past decade on the relationship between tyrosine availability and catecholamine synthesis. It also compares this relationship with that of tryptophan to serotonin. (Interactions between acetylcholine, choline, and phosphatidylcholine have recently been reviewed elsewhere [10]).

EARLY STUDIES RELATING TYROSINE TO CATECHOLAMINE PRODUCTION

The failure of tyrosine administration to increase brain levels of its neurotransmitter products had led virtually all investigators to assume that tyrosine hydroxylase was fully saturated with its amino acid substrate *in vivo*—this in spite of the fact that estimates of the enzyme's K_m for tyrosine *in vitro* [50–125 μM, depending upon whether tetrahydrobiopterin or a synthetic alternate was used as the cofactor (e.g. Refs. 11 and 12)] were not very much lower than whole-brain tyrosine levels [which vary between 100 and 200 μM, depending on the protein content of the meal most recently consumed (Table 1)]. Catecholamines, however, were known to be stored within multiple metabolic compartments, including some with slow turnover times; this raised the possibility that supplemental tyrosine might actually accelerate the synthesis of a particular "pool" of dopamine or norepinephrine, but that this pool constituted too small a fraction of the total catecholamine store to allow detection. Hence, experiments were performed to determine whether changes in tyrosine levels might affect rates of catecholamine *synthesis*, as estimated from the accumulation of dihydroxyphenylalanine (DOPA) in brains of animals pretreated with a decarboxylase inhibitor [7, 15]. Under such conditions, DOPA accumulation was shown to be accelerated when brain tyrosine levels were increased (by giving rats tyrosine), and diminished when tyrosine was reduced (by giving rats other large neutral amino acids, like tryptophan, valine, or parachlorophenylalanine, which compete with tyrosine for passage across the blood–brain barrier [23]). Such observations provided estimates of the K_m for the hydroxylation *in vivo* of tyrosine and showed that this process could indeed be affected when tyrosine levels varied within their normal range. However, the use of a decarboxylase inhibitor—which also diminishes catecholamine synthesis in nerve terminals—rendered problematic the extrapolation of these data to physiologic states, since the drug probably also reduced both the end-product inhibition of tyrosine hydroxylase and the release of newly-formed catecholamine into synapses. What was needed was an experimental approach that allowed catecholamine synthesis to be estimated, in the presence of varying brain tyrosine concentrations, without concurrently disturbing that synthesis. When one such approach was tried—that of measuring dopamine metabolites in striata of animals given tyrosine—no effect of tyrosine was observed [24]. (Tissue dopamine levels were subsequently shown to rise in striata and cortices of rats given

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Table 1. Tyrosine levels in tissues and fluids

Species	Tissue or fluid	[Tyrosine]	Comments	Ref
Rat	Brain	47–65 nmoles/g	Fasted	13, 14
Ret	Brain	47–173 nmoles/g	Non-fasted	13–15
Rat	Plasma	70–80 μM	Adult	16, 17
Rat	Plasma	188 ± 21 μM	Neonatal	18
Rat	Plasma	233 ± 2 μM	Fetal	18
Dog	CSF	16 ± 3 μM		18
Primate	CSF	4–5 μM	Males, females	19
Human	CSF	8–10 μM	Normal	18, 20, 21
Human	CSF	19–20 μM	Parkinsonian	22
Human	Plasma	50–60 μM	Normal, fasting	20, 21

large doses of tyrosine methyl-ester [25].) However, if the animals were concurrently given haloperidol, a dopamine receptor antagonist that accelerates nigrostriatal firing [26] and can lower tyrosine levels in striata [27], catecholamine production did exhibit precursor dependence: striatal levels of homovanillic acid (HVA) varied directly with those of brain tyrosine, while dopamine levels themselves remained constant [24]. These observations were interpreted as indicating that a given catecholaminergic neuron might or might not be responsive to having more or less tyrosine, depending on its level of activity.

The ability of tyrosine supplementation to enhance the synthesis of catecholamines in, and their release from, rapidly firing neurons (but not from relatively quiescent cells) has since been affirmed using a variety of experimental manipulations (Table 2). Thus, tyrosine administration increases brain levels of the norepinephrine metabolite methoxy-hydroxy-phenylethylgylcol sulfate (MHPG-SO_4) in cold-stressed rats [30] and in brains and brainstems of spontaneously hypertensive rats (SHRs) [31, 32] but not in those of control, normotensive animals, nor in SHRs given both tyrosine and another large neutral amino acid, valine [31], which competes with tyrosine for uptake into the brain [23]. Increases in MHPG-SO_4 after tyrosine treatment have also been observed in rats given yohimbine [34], an alpha$_2$ antagonist, or in animals stressed by tail-shock [36]. DOPA accumulation (after decarboxylase inhibition) is accelerated following tyrosine administration in striata of rats given gamma-butyrolactone [29] (which blocks the release of dopamine and the consequent activation of presynaptic inhibitory autoreceptors), and in the median eminence of animals that receive exogenous prolactin [35] (which presumably activates the tuberoinfundibular dopaminergic neurons via a short feedback loop). Following a lesion that destroys about 80% of the nigrostriatal tract unilaterally (and thus accelerates the firing of the surviving neurons [38]), tyrosine administration increases dopamine release on the lesioned side, as estimated from the ratios of dihydroxyphenylacetic acid (DOPAC) or HVA to dopamine, or to tyrosine hydroxylase activity, but fails to affect either index on the intact side [28]. The tyrosine effect is, once again, blocked by valine, and is unassociated with changes in dopamine levels. Tyrosine administration also increases brain levels of dopamine metabolites in animals pretreated with reserpine [33], amfonelic acid, or spiperone [37], all of which, like haloperidol, are thought to accelerate nigrostriatal firing. In meso-

Table 2. Tyrosine administration and catecholamine synthesis and release

Tissue	Treatment	Biochemical index	Tyrosine effect (%)	Ref.
Striatum	Haloperidol	DOPAC, HVA	+60	24
Striatum, Limb. forb.	Haloperidol	DOPA	+15	15, 27
Striatum	NS tract lesions	DOPAC, HVA	+60	28
Striatum	γ-butyrolactone	DOPA	+25	29
Whole brain	Cold stress	MHPG-SO_4	+70	30
Whole brain	SHRs	MHPG-SO_4	+40	31
Brainstem, forebrain	SHRs	MHPG-SO_4	+15	32
Striatum, hypothalamus	Reserpine	DOPAC, HVA	−40	33
Whole brain	Yohimbine	MHPG-SO_4	−35	34
Med. eminence	Prolactin	DOPA	−30	35
Hippocampus, hypothalamus	Tail shock	MHPG-SO_4	−40	36
Whole brain	Amfonelic acid, spiperone	DOPAC	+30	37

prefrontal dopamine neurons which have been shown to have a high physiological firing rate and exhibit a lot of bursting activity, low doses of tyrosine are effective in increasing dopamine synthesis [39]. Tyrosine increases levels of dopamine metabolites in light-activated rat retinas *in vivo* [40], but not when animals are in darkness.

TYROSINE, NIGROSTRIATAL FIRING, AND DOPAMINE RELEASE

Direct evidence that physiologic variations in tyrosine availability can affect dopamine release was obtained recently using an experimental system in which superfused slices of rat striatum were subjected to electrical pulses (20 Hz, 2 msec) of varying train length; the amount of endogenous dopamine released into the medium was correlated with its tyrosine concentration (Figs. 1 and 2). Tissues were subjected to two trains of 600 or 1800 pulses, each lasting 30 or 90 sec, respectively, and separated by 30 min; dopamine release during the second (S_2) period is expressed as a decimal fraction of the amount released during the initial (S_1) period. When slices were superfused with Krebs bicarbonate buffer (which lacks tyrosine or any other amino acid), the S_2/S_1 ratio was 0.75–0.80, depending on the number of pulses (that is, dopamine release during S_2 declined by 20–25%). A tyrosine concentration in the superfusate of at least 20 μM was needed to maintain an S_2/S_1 ratio of unity in slices stimulated for 30 sec, while at least 40 μM was needed in tissues stimulated for 90 sec. Tissues that have been stimulated while superfused with the tyrosine-free buffer display major reductions in tyrosine content (up to 50%), as well as in dopamine itself (25%) [41, 42].

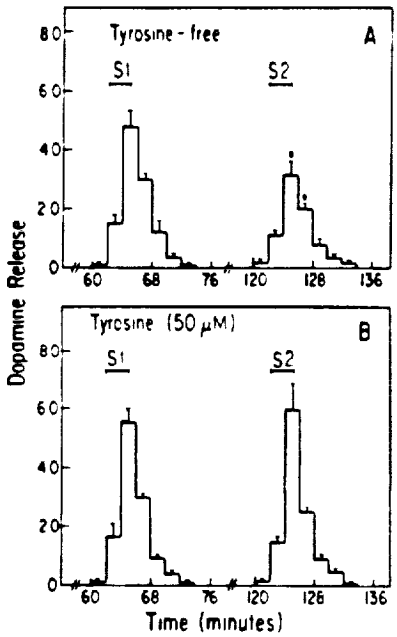

Fig. 1. Release of endogenous dopamine evoked by electrical stimulation from rat striatal slices, expressed as percentage released of final tissue content. (A) tyrosine-free medium; (B) tyrosine-supplemented medium (50 μM). S_1 and S_2 were identical trains of 1800 pulses (60 mA, 2 msec, 20 Hz) delivered 60 min apart. Superfusate was collected in 2-min fractions and assayed for dopamine by alumina extraction and high performance liquid chromatography-electrochemical detection. Data were analyzed by paired Student's *t*-tests, and values shown are mean ± S.E.M. for four experiments. (*) $P < 0.05$ when compared to equivalent fraction from S_1. (Data from Ref. 41.)

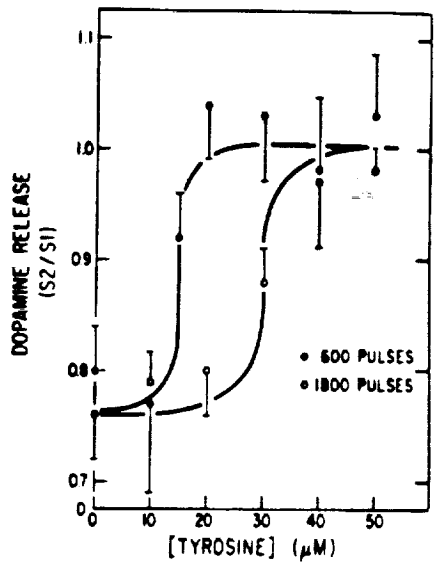

Fig. 2. Effect of tyrosine on the stimulus-evoked release of endogenous dopamine from superfused rat striatal slices. Trains of electrical pulses (60 mA, 2 msec, 20 Hz) were delivered for 30 (600 pulses) or 90 (1800 pulses) sec. Dopamine release during S_1 averaged 3.92 ± 0.26 pmoles/mg (600 pulses) and 7.91 ± 0.59 pmoles/mg (1800 pulses), thus declining in the amount released per pulse by approximately 33%. Total dopamine released during the first train (S_1) was compared to that released by a second identical train applied 30 min later (S_2/S_1). Tyrosine dose-dependently increased the release of dopamine during S_2, with S_2/S_1 approximating unity at tyrosine concentrations of 20 μM (600 pulses) or 40 μM (1800 pulses). Comparison of S_2/S_1 ratios for the two trains by Student's *t*-tests showed significant differences ($P < 0.05$) in dopamine release evoked by S_2 during superfusion with 20 and 30 μM tyrosine. Each point represents the mean ± S.E.M. of four to seven animals. (Data from Ref. 42.)

Since dopaminergic terminals comprise only a small percentage of the total cellular mass of the striatum, this major depletion of striatal tyrosine suggests either that the amino acid becomes depleted within non-catecholaminergic cells, as well as within these terminals, or that most of the tyrosine normally present in the striatum is confined within dopaminergic terminals. In experiments designed to examine the latter possibility, these terminals were destroyed unilaterally by injecting the neurotoxin 6-hydroxydopamine into the substantia nigra. Even though the dopamine content of the ipsilateral striatum was depleted by more than 95%, its tyrosine levels were unchanged, indicating that striatal tyrosine is not preferentially localized within the dopaminergic terminals, and suggesting that the tyrosine depletion that occurred when the superfused slices were stimulated reflected mobilization of the amino acid from non-dopaminergic as well as from dopa-

minergic cells (J. D. Milner, D. K. Reinstein and R. J. Wurtman, unpublished data). *In vivo*, dopaminergic nerve terminals are, of course, perfused not with a tyrosine-free solution but with tyrosine-containing blood; moreover, as discussed below, circulating tyrosine is able to enter the brain, its entry catalyzed by a facilitated diffusion system that it shares with other large, neutral amino acids [23]. Hence it would not be expected that even the prolonged conversion of tyrosine to dopamine would cause its depletion, at least to the extent seen *in vitro*. However, the rate at which tyrosine diffuses from the plasma into dopaminergic nerve terminals is retarded by both the limited water-solubility of the amino acid and the competition between it and other circulating amino acids for attachment to the blood–brain barrier transport site [23] and to neuronal membranes [43]. Hence, the possibility remains that tyrosine in nerve terminals may fall, after prolonged neuronal firing, to levels sufficient to slow catecholamine synthesis. In that circumstance the ability of supplemental tyrosine to enhance catecholamine synthesis would be explained not so much by its ability to increase the substrate-saturation of tyrosine hydroxylase but by *blocking the decrease* that would otherwise occur. The inability of striatal dopaminergic terminals to sustain transmitter output without exogenous tyrosine contrasts with the ability of cholinergic neurons in the same tissue to continue making their neurotransmitter even when exogenous choline is lacking. Cholinergic terminals continue to release unchanged amounts of acetylcholine, even after 30 min of continuous stimulation, when superfused with the Krebs solution (which also, of course, lacks choline) [44]. The source of choline for this acetylcholine synthesis is probably a "reservoir" in the form of membrane phosphatidylcholine (PC) [45]; the PC is hydrolyzed to free choline, which is then released into the extracellular space and then taken back up into the cholinergic terminal for acetylation. Apparently the protein in nerve terminals is unable to serve in an analogous manner as a reservoir for tyrosine.

TYROSINE AND SYMPATHO-ADRENAL CELLS

Tyrosine availability has also been shown to affect catecholamine synthesis in peripheral tissues. Its acute or chronic (8 days) administration to cold-exposed rats caused dose-related increases in urinary norepinephrine and epinephrine [46]; valine or leucine failed to elicit similar responses, and, when administered with tyrosine, blocked the increases [47]. The increase in urinary epinephrine was also blocked by bilateral adrenalectomy [48]. In contrast, the rise in urinary norepinephrine was amplified in rats whose sympathetic terminals had been partially destroyed by prior administration of 6-hydroxydopamine [48], suggesting that an increase in their firing rates had occurred, rendering them more responsive to the amino acid. Tyrosine administration also caused increases in urinary dopamine; these were unaffected by adrenalectomy or 6-hydroxydopamine. Administration of oral tyrosine (33 mg/kg prior to each meal) to human subjects also increased urinary levels of the three catecholamines [49]. After a single dose of the amino acid (100 or 150 mg/kg), urinary levels of the catecholamines and their principal metabolites all increased, with time-courses that paralleled the rise in blood tyrosine levels [50]. These observations were interpreted as suggesting that, in humans, both central and sympathoadrenal catecholamine synthesis are precursor-responsive.

Enhancement of sympathoadrenal catecholamine synthesis underlies the ability of tyrosine to restore blood pressure in rats in hemorrhagic shock [51]. This response is blocked by bilateral adrenalectomy, performed immediately prior to testing [51], or by pretreatment with carbidopa or phentolamine [52], and is not simulated by other large neutral amino acids (like valine or leucine) [52]. (Tyrosine administration to hypotensive rats also elevates levels of epinephrine in the adrenal medulla, and of norepinephrine in the spleen [52].) That the pressor effect of tyrosine is not mediated by its conversion to the sympathomimetic amine tyramine was demonstrated by its lack of effect (unlike tyramine) in rats receiving a ganglionic blocker (hexamethonium), its persistent pressor activity (again unlike tyramine) in reserpinized hypotensive rats, and its failure to elevate plasma tyramine levels when raising blood pressure [53].

The fact that a given dose of tyrosine can raise blood pressure in hypotensive animals, lower it in spontaneously-hypertensive rats, and have little or no effect in normotensive animals (or people) has been interpreted as resulting from the ability of tyrosine to enhance catecholamine synthesis only in neurons (or chromaffin cells) undergoing prolonged physiological activity: in hypotensive rats, the sympathoadrenal cells are active, and thus tyrosine-responsive, hence giving the amino acid potentiates their release of catecholamines, restoring blood pressure. In SHR, these peripheral neurons may be negatively quiescent, suppressed by the now-active brain-stem noradrenergic neurons that control sympathetic outflow [54]; hence, tyrosine administration, by enhancing norepinephrine synthesis *within* the brain stem, further reduces sympathetic activity, causing blood pressure to fall. The possible utility of oral tyrosine in treating hypertension is currently under investigation [55]; its use in treating shock (which would require parenteral administration) is hampered by its very poor water-solubility.

PLASMA AND TISSUE TYROSINE

Published data on the levels of tyrosine in various tissues and body fluids are summarized in Table 1 [16–22]. Tyrosine recently taken up from the extracellular space may be used preferentially for dopamine synthesis [56] (i.e. in contrast to tyrosine already present in the cytoplasm). This would allow acute changes in the plasma amino acid pattern to have relatively more of an effect on the synthesis and release of this neurotransmitter. The constituents of the plasma that affect brain tyrosine are not only the amino acid itself, but also the other large, neutral amino acids (LNAA) (primarily tryptophan, phenylalanine, valine, isoleucine, and leucine) that compete with tyrosine for entry into the brain [17, 23]. Brain

tyrosine levels sometimes correlate only poorly with plasma tyrosine concentrations (e.g. after a protein-rich meal) but apparently are always well-correlated with the "plasma tyrosine ratio" (to other LNAA) [13]. Hence, an increase in plasma levels of the branched-chain amino acids—such as would result from insulin deficiency or insensitivity—could decrease both the transport of tyrosine across the blood–brain barrier, and its subsequent conversion to catecholamines. Both dietary [13, 14] and pharmacological [57] manipulations of plasma LNAA levels have been shown to cause the predicted changes in brain tyrosine levels. Probably the most effective way to increase brain tyrosine is to administer the amino acid orally, along with sufficient carbohydrate to elicit insulin secretion and, thereby, to lower plasma levels of the other LNAA [58].

Recent studies have obtained evidence that a variety of severe stresses (e.g. immobilization [59], hemorrhage [60]) can selectively increase the plasma tyrosine ratio. This may reflect a shunting of blood away from the liver, where tyrosine is metabolized, and could serve to increase the availability of the amino acid.

FIRING FREQUENCY, TYROSINE-DEPENDENCE, AND ALLOSTERIC CHANGES IN TYROSINE HYDROXYLASE

An additional mechanism by which increased activity can cause catecholaminergic neurons to become tyrosine sensitive involves kinetic changes in tyrosine hydroxylase that result from its phosphorylation. This process is accelerated when neuronal activity increases. Phosphorylation of tyrosine hydroxylase can be catalyzed by any of several protein kinases, each of which acts selectively on particular amino acids in the enzyme protein [61, 62].

The enzyme activation occurring when neuronal activity increases (i.e. after *in vitro* depolarization of rat striatum) is dependent on calcium and calmodulin [63]. It increases the activity of the enzyme, without changing its affinity for tyrosine or the tetrahydrobiopterin cofactor, nor its susceptibility to end-product inhibition. A different, cAMP-dependent, protein kinase can also phosphorylate tyrosine hydroxylase, increasing its affinity for its cofactor (but not tyrosine), and also perhaps decreasing its susceptibility to end-product inhibition [64, 65]. Recent evidence suggests that, in striatal dopaminergic nerve terminals, the dopamine released following depolarization acts via presynaptic autoreceptors to decrease local cAMP levels; this slows the phosphorylation of the tyrosine hydroxylase and decreases its activity [66]. Hence, the physiologic role of the cAMP-dependent protein kinase in striatum may be to *suppress* the hydroxylation of tyrosine in response to prolonged dopamine release. In contrast, the calcium–calmodulin-dependent protein kinase is activated when voltage-gated calcium channels open during membrane depolarization; catecholamine formation thereupon depends on the extent to which the tyrosine hydroxylase happens to be saturated with tyrosine. This, in turn, will depend on the K_m of the enzyme for tyrosine (which apparently does not change with phosphorylation), and on tyrosine levels within the nerve terminals. The latter have not been measured, but presumably they bear a relationship to whole-brain tyrosine levels (which, in turn, vary with the plasma tyrosine ratio, as discussed above).

PHYSIOLOGICAL CONSEQUENCES OF TYROSINE ADMINISTRATION

If tyrosine availability does indeed affect brain catecholamine synthesis and release, it should be expected that it will also influence various behaviors and physiological processes that involve catecholaminergic neurotransmission. Some publications demonstrating such effects are described in Table 3 [67–72]. One such catecholamine-dependent process is the control of blood pressure, which is *elevated* by the release of norepinephrine or epinephrine from sympathoadrenal cells, and either decreased or increased by norepinephrine release within the central nervous system, depending on the locus of this release [54]. In SHRs, tyrosine injection causes a marked fall in blood pressure, which is blocked by coadministration of other LNAAs [31]. Under these conditions, brainstem levels of the major norepinephrine metabolite MHPG-SO_4 are elevated. Similar findings are obtained when tyrosine

Table 3. Effects of tyrosine on catecholamine-mediated phenomena

Animal model	Physiological parameter	Tyrosine effect	Ref.
Rat	Open field behavior	Reversal of stress-induced inhibition	67
SHRs	Blood pressure	Hypotensive	31, 32, 55
Rat (hemorrhaged)	Blood pressure	Hypertensive	51
Dog	Ventricular arrhythmia	Preventative	68
Rat	Renal hypertension	Hypotensive	69
Aged, anestrous female rats	Estrous cycling	Restored	70
Aged mice	Motor activity	Increased	71
Mice	Swim test immobility	Ameliorated	72
Mice	Open field behavior	Increased activity	72

is injected into the lateral ventricles of these animals [32]. These observations indicate a central mechanism for the antihypertensive effect of tyrosine (consistent, for example, with enhanced norepinephrine release from locus coeruleus neurons). Tyrosine administration to stressed rats also causes behavioral effects; when given via the diet prior to stress or by injection thereafter, it blocks the stress-induced fall in regional brain norepinephrine levels and amplifies the increases in MHPG-SO_4; it also blocks such post-stress behavioral changes as diminished locomotor activity and a diminished tendency of the animal to manifest its "curiosity" by standing on its hind legs or poking its nose into a hole [36, 67]. Attempts to determine whether tyrosine may also affect behavior in healthy humans or in those with psychiatric disease are in their infancy.

COMPARISON OF THE EFFECTS OF TYROSINE AND TRYPTOPHAN ON THE SYNTHESES OF THEIR NEUROTRANSMITTER PRODUCTS

Although the syntheses of the catecholamine neurotransmitters and of serotonin are both dependent on available concentrations of their amino acid precursors (tyrosine and tryptophan), and although both biosynthetic pathways are catalyzed by specific, low-affinity hydroxylase enzymes that use tetrahydrobiopterin as their cofactor, the mechanisms that actually couple physiologic changes in tyrosine or tryptophan levels to formation of their transmitter products differ in important ways. The consumption of traditional foods (i.e. those not designed specifically to affect brain amino acids) can cause major changes in brain tryptophan levels, and thus in serotonin synthesis; in contrast, it is necessary to consume pure tyrosine, separate from the other amino acids in dietary proteins, in order to obtain major increases in brain tyrosine levels, or to administer the other LNAA in order to reduce brain tyrosine. (This is because tryptophan is scarce in dietary proteins and relatively unaffected by the insulin secreted by dietary carbohydrates, while tyrosine, like the other LNAA, is both more abundant in proteins and more affected by insulin.) Another major difference concerns the extent, discussed above, to which neurons must undergo sustained physiological activity in order for them to respond to supplemental tyrosine or tryptophan. Serotonin-releasing neurons will virtually always produce more or less of their transmitter when they are provided with more or less of its precursor; catecholamingeric neurons are responsive to tyrosine when they have been very active, but, in general, unresponsive in other circumstances. These differences allow serotoninergic neurons to functions as "sensors" of the nutrient content of the diet, whereas the effects of tyrosine presently appear to be more in the realm of pharmacology, sustaining and even amplifying transmitter release in response to increased activity of catecholaminergic cells.

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